



DOCKET NO. 13131-0310 (44378-282108)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)
)
Cham et al.)
) Art Unit: 1648
Serial No. 10/601,656)
) Examiner: Stacy B. Chen
Filed: June 20, 2003)
)
For: A Method of Treating and Preventing)
Infectious Diseases Via Creation of a Modified)
Viral Particle With Immunogenic Properties)

DECLARATION OF MR. HASSIBULLAH AKEEFE UNDER 37 C.F.R. §1.132

I, Hassibullah Akeefe, B.Sc., do hereby declare:

1. I am one of ordinary skill in the art in the field of lipid studies. I am currently a Scientist/Research Lab Manager at Lipid Sciences Inc., Pleasanton, California. I earned a B.Sc. degree in Biochemistry in 1994 at the University of Maryland at College Park, Maryland. My *curriculum vitae* is enclosed (Exhibit A). The list of the publications is enclosed (Exhibit B).

2. I am familiar with U.S. Patent Application Serial No. 10/601,656 ("the present application") and U.S. Patent No. 5,419,759 to Naficy (hereinafter "*Naficy*").

3. I declare that enclosed herewith as Exhibit C are the data obtained by a laboratory at Johns Hopkins University, Baltimore, Maryland, which was contracted by assignee of the present application, Lipid Sciences, Inc., to examine the effect of delipidation on structural integrity of immunodeficiency virus particles. These data are electron micrographs (EM) that characterize the results of delipidation of human immunodeficiency virus (HIV) particles using various solvent concentrations. The control electron micrograph (Fig. 1) shows viral particles not exposed to organic solvent. These control viral particles (arrows) exhibit an electron dense viral core surrounded by a noticeable and well defined

U.S. Patent Application Serial No. 10/601,656

Declaration Under 37 C.F.R. §1.132

Page 2

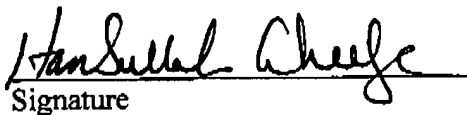
envelope. Delipidation by 1% diisopropyl ether (DIPE) resulted in partially delipidated viral particles (arrows) similar to the control viruses (Fig. 2). Delipidation by 1% butanol resulted in viral particles (arrows) comprising a denser core than the control viruses (Fig. 3). Delipidation by a combination of 1% butanol/DIPE resulted in partially delipidated viral particles with a denser core than the control viruses and partial destruction of viral envelope (arrows; Fig. 4). Delipidation by 2% butanol resulted in viral particles with a denser core than delipidation with 1% solvent and partial destruction of viral envelope.(arrows; Fig. 5). Delipidation by 5% butanol resulted in destruction of viral envelopes and obliteration of viral integrity and only electron dense cores remained (arrows; Fig. 6). Thus, as the viral particles were delipidated with increasing solvent concentrations, the lipid envelope was compromised or destroyed. These micrographs reflect the destruction of the viral envelope comprising envelope-associated proteins and destruction of partially delipidated immunogenic viral particles upon delipidation with 5% solvent.

4. As one of ordinary skill in the art, I declare that *Naficy* teaches the disappearance of viral infectivity upon delipidation. *Naficy* shows that the delipidation conditions result in destruction of viral particle integrity. *Naficy* teaches **no recovery of infectivity of "up to 7 logs of virus"** after incubation with 5% diethyl ether at room temperature for 5 minutes. See *Naficy*, Column 9, lines 10-11, 15-17, and 33-35. In contrast, the method of the present application results in **a 2.5 log reduction in infectivity upon delipidation, with the remaining virus titer of $10^{4.5}$** . See the present application, p. 53, lines 16-17. The delipidation of immunodeficiency virus by the method of the present application results in a reduction, rather than complete disappearance of infectivity. In contrast, *Naficy* teaches disappearance of viral infectivity, indicating destruction of viral particle integrity. It is my opinion that, **upon delipidation, the plasma in *Naficy* would not contain immunogenic partially delipidated viral particles**. Applicants or I did not perform the experiments described in *Naficy* and did not assess the integrity of the delipidated HIV products obtained by the methods taught in *Naficy*. However, the electron micrographs provided herewith as

U.S. Patent Application Serial No. 10/601,656
Declaration Under 37 C.F.R. §1.132
Page 3

Exhibit C demonstrate that delipidation using 5% or more solvent, as taught in *Naficy*, would result in destruction of viral particle integrity.

5. I declare further that all statements made herein are of my own knowledge and are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of any patent issuing on this application.


Signature

Hassibullah Akeefe

Name

February 27, 2006

Date



Hassibullah Akeefe

Experience:

12/2005 – Present Scientist/Research Lab Manager Lipid Sciences Inc. Pleasanton, CA

Manage R&D laboratory activities regarding research and development activities in both the selective HDL delipidation and Viral Immunotherapy platforms. Manage scientists and research associates to further develop, optimize and characterize the effects of delipidation on plasma lipoproteins and biochemistry as well as developing new and novel methods for creating vaccines for the Viral Immunotherapy Program. Execute and manage R&D laboratory and personnel in support of the human clinical trial.

6/2002 – 12/2005 Research Associate II Lipid Sciences Inc. Pleasanton, CA

Characterization and analysis of plasma lipids and protein in development of the companies selective delipidation of Plasma lipoprotein patent pending process. Development of methods for generating antigenic viral particles for HIV, SIV and SARS. In vitro and in vivo analysis and characterization of these particles. Development and characterization of a delipidation protocol for the generation of highly antigenic particles for the treatment of cancers. Assisted in the development, design, testing and validation of the delipidation system.

10/1999 - 6/15/2002 Research Associate III Avigen Inc. Alameda, CA

Characterization, construction, production, purification and analysis of various Adeno-Associated Virus (AAV) vectors in a cell based system; generation of stably transfected cell lines; work on analyzing the feasibility of various AAV serotypes in production of clinical vectors for the treatment of disease.

9/1995 - 10/1999 Research Associate I J. David Gladstone Institutes San Francisco, CA

Screening, generation and maintenance of transgenic mouse and rabbit colonies; microdissection, fixation, sectioning and immunohistochemical staining of tissue; DNA/RNA isolation; Southern, Northern and Western blotting; ELISA; generating transgenic mouse hippocampal cDNA library; minor animal surgery; managing and organizing of the lab.

7/1997 - 10/1999 Staff Research Associate
Children's Hospital Oakland Research Institute Oakland, CA

Screening, generation and maintenance of transgenic mice; characterization and analysis of transgenic mouse plasma using FPLC, purification and generation of antibodies specific for

hepatic lipase; developing screening and analysis assay's (PCR, qPCR, ELISA) for the research project; management and organization of the lab supplies.

9/1994 - 6/1996 Laboratory Technician Lifescan Inc. Milpitas, CA

Testing, analysis, manufacturing, and quality control of the company's glucose strips according to the company's cGMP and cGLP guidelines.

Education:

12/1994 University of Maryland Bachelor's Degree College Park, MD

Technical Skills:

- Eukaryotic cell culture; immortalized lines and primary lines
- Bacterial culture and transformation
- Adenovirus preparation, purification, and quantification
- Molecular cloning and recombinant DNA techniques
- Southern, Northern and Western blotting and hybridization
- Small animal handling experience
- Nucleic Acid and Protein purification
- PCR/RT-PCR/qPCR
- FACS
- Column Chromatography
- GC
- Certified Hitachi 911
- Adeno-Associated Virus preparation, purification and quantification.
- Tissue fixing/ sectioning and staining.



Publications:

1. Moiz Kitabwalla, Francois Villinger, Aftab A. Ansari, James E.K. Hildreth, Hassibullah Akeefe, Zhaohao Liao, Ann E. Mayne, Lisa Gargano, Adam P. Conner, Jo-Ann Maltais, Gretchen Kunas, and Marc Bellotti. Enhancement of cell mediated immune responses using lipid depleted lentivirus as immunogen: A novel approach for inducing recognition of new viral epitopes. *Vaccine*. (23). pp4666-4677, 2005.
2. H. Dichek, W. Brecht, J. Fan, Z.S Ji, S. McCormick, H. Akeefe, L. Conzo, D. Sanan, K. Weisgraber, S. Young, J.M Taylor and R.W Mahley. Overexpression of Hepatic Lipase in Transgenic Mice Decrease Apolipoprotein B-containing and High Density Lipoproteins: Evidence that Hepatic Lipase Acts as a Ligand for Lipoprotein Uptake. *Journal of Biological Chemistry*, Jan 1998 273(4): 1896-1903
3. M. Buitini, M. Orth, S. Bellosta, H. Akeefe, R.E Pitas, T.W Corray, L. Mucke and R.W Mahley. Expression of Human Apolipoprotein E3 or E4 in Neurons of ApoE Knockout Mice: Isoform-specific Effects on Age-related Neurodegeneration In Vivo, *Journal of Neuroscience*, Jun 1998 18(12): 4867-80
4. M. Buitini, H Akeefe, C. Lin, R.W Mahley, R.E Pitas, T.W Corray, and L. Mucke. Dominant Negative Effect of apolipoprotein E4 revealed in transgenic models of neurodegenerative disease, *Journal Neuroscience* 1999 JUN 15; 19(12):4867-80
5. H. L. Dichek, S. M. Johnson, H. Akeefe, G. T. Lo, E. Sage, C. E. Yap, and R. W. Mahley. Hepatic lipase overexpression lowers remnant and LDL levels by a noncatalytic mechanism in LDL receptor-deficient mice. *J. Lipid Res.*, February 1, 2001; 42(2): 201 – 210

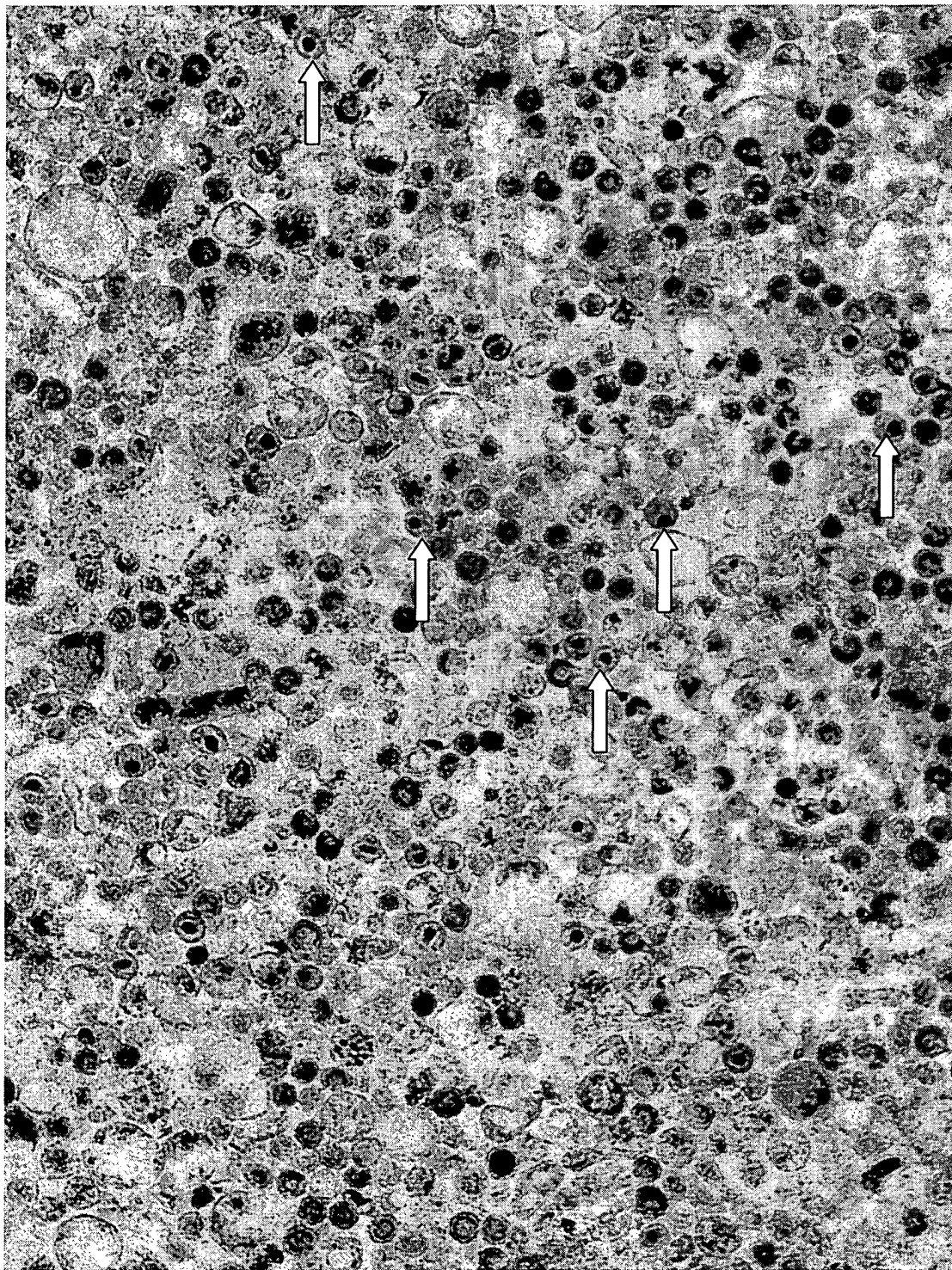
Abstracts and Presentations:

1. Inhibition of AAV Vector Transduction by Animal Sera In Vitro Dorothy Huey-Louie,¹ James Allen,¹ Hassibullah Akeefe,¹ Brian Christie,¹ Shang-Zhen Zhou,¹ Richard Surosky,¹ Jennifer Wellman,¹ Alan McClelland,¹ Peter Colosi.¹
2. A Simple, Efficient, and General Method for the Production of AAV Type 1-6 Vectors Shang-Zhen Zhou,¹ Brian Christie,¹ Jennifer Wellman,¹ James Allen,¹ Hassibullah Akeefe,¹ Richard Surosky,¹ Michael Lochrie,¹ Dirk Grimm,² Clare Thomas,² Hiroyuki Nakai,² Mark Kay,² Alan McClelland,¹ Peter Colosi.¹
3. Neutralizing Activity Against Different AAV Serotypes in Sera from Untreated Humans and in Sera from Humans Treated with an AAV2 Vector Richard Surosky,¹ Dorothy Huey-Louie,¹ James Allen,¹ Shang-Zhen Zhou,¹ Brian Christie,¹ Hassibullah Akeefe,¹ Ciaran Scallan,¹ Sharon Powell,¹ Linda Couto,¹ Katherine High,² Mark Kay,³ Alan McClelland,¹ Peter Colosi.¹

4. Benefit of Delipidated Retroviruses as Potential Autologous Therapeutic Vaccines. AIDS Vaccine 2005 International Conference. Montreal, Quebec. September 6-9, 2005. Abstract#107P.M. Kitabwalla¹, H. Akeefe¹, A. Ansari², F. Villinger², A. Conner¹, J.E.K. Hildreth³, and M. Bellotti¹. Lipid Sciences, Inc., Pleasanton, CA¹; Emory University School of Medicine, Atlanta, GA²; Johns Hopkins School of Medicine, Baltimore, MD³
5. Delipidated Retroviruses as Potential Autologous Therapeutic Vaccines-A Pilot Experiment. Keystone Symposia on HIV Vaccine Development. Banff, Alberta. April 9-15, 2005. Abstract#237. M. Kitabwalla³, A. Ansari¹, F. Villinger¹, H. Akeefe³, A. Conner³, J.E.K. Hildreth², and M. Bellotti³. Emory University School of Medicine, Atlanta, GA¹; Johns Hopkins School of Medicine, Baltimore, MD²; Lipid Sciences, Inc., Pleasanton, CA³
6. Solvent-Treated Retroviruses as Novel Vaccines-A Study in Characterizing Delipidated Retroviruses. Keystone Symposia on HIV Vaccine Development. Banff, Alberta. April 9-15, 2005. Abstract# 227. J.E.K. Hildreth¹, Z. Liao¹, H. Akeefe², A. Conner², M. Bellotti², A. Ansari³, F. Villinger³, and M. Kitabwalla². Johns Hopkins School of Medicine, Baltimore, MD¹; Lipid Sciences, Inc., Pleasanton, CA²; Emory University School of Medicine, Atlanta, GA³.
7. A Prime-Boost Immunization Strategy Using Delipidated SIV Gives Rise to a Broader CD4+ and CD8+ T-Cell Responses in Mice than AT-2 Treated or Live Virus-A Novel Therapeutic Vaccine Approach for HIV Infection. Keystone Symposia on HIV Vaccine Development. Whistler, B.C. April 11-18, 2004. Abstract#304. Ansari¹, F. Villinger¹, J. E.K. Hildreth², M. Bellotti³, J. B. Maltais³, H. Akeefe³, T. Perlman³, A. Conner³, G. Kunas³, and M. Kitabwalla³. Emory University School of Medicine, Atlanta, GA¹; Johns Hopkins School of Medicine, Baltimore, MD²; Lipid Sciences, Inc., Pleasanton, CA³.

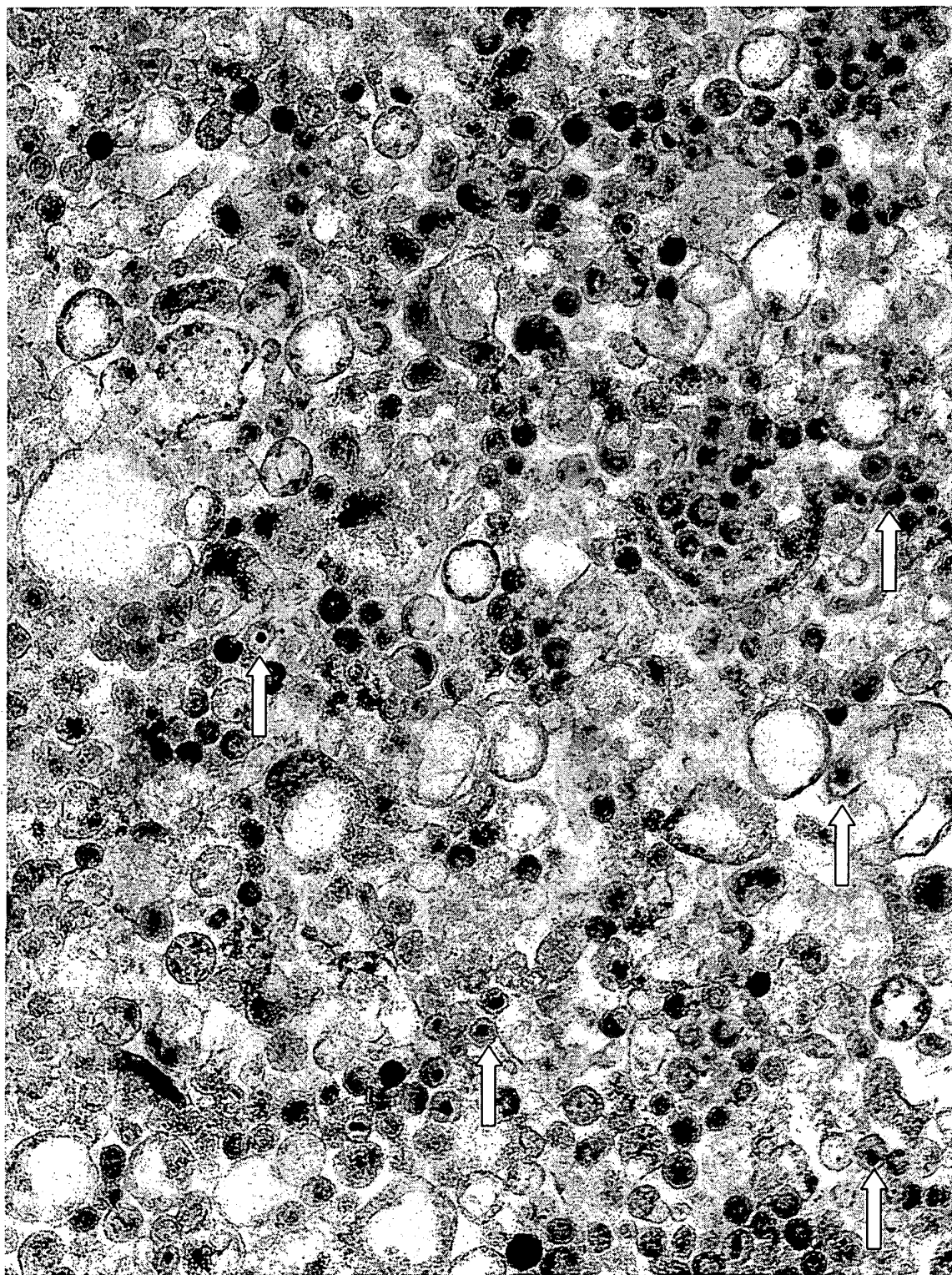


EM Analysis (HIV-1RF): Control
Figure 1



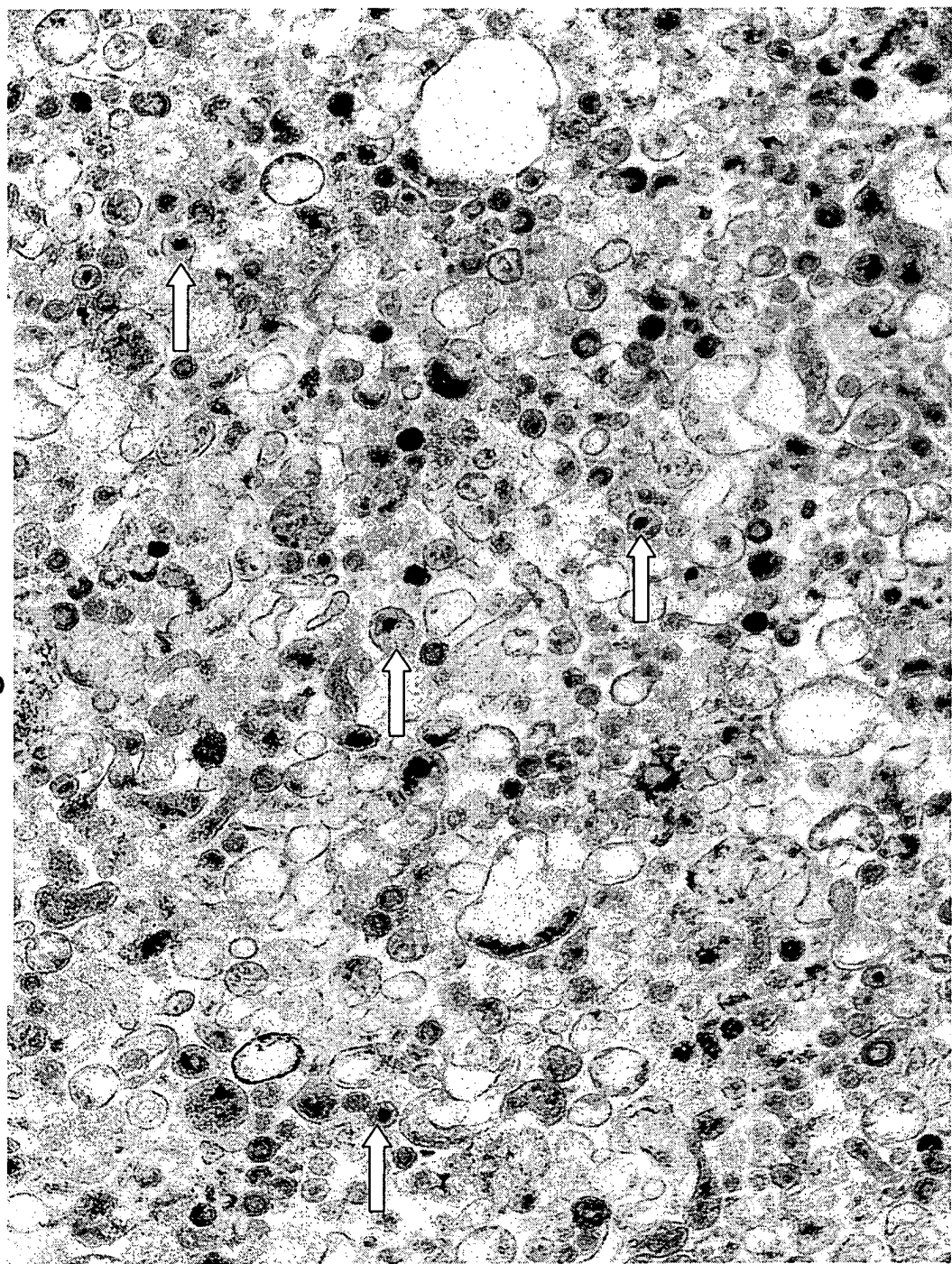


EM Analysis HIV-1RF: 1% DIPE
Figure 2

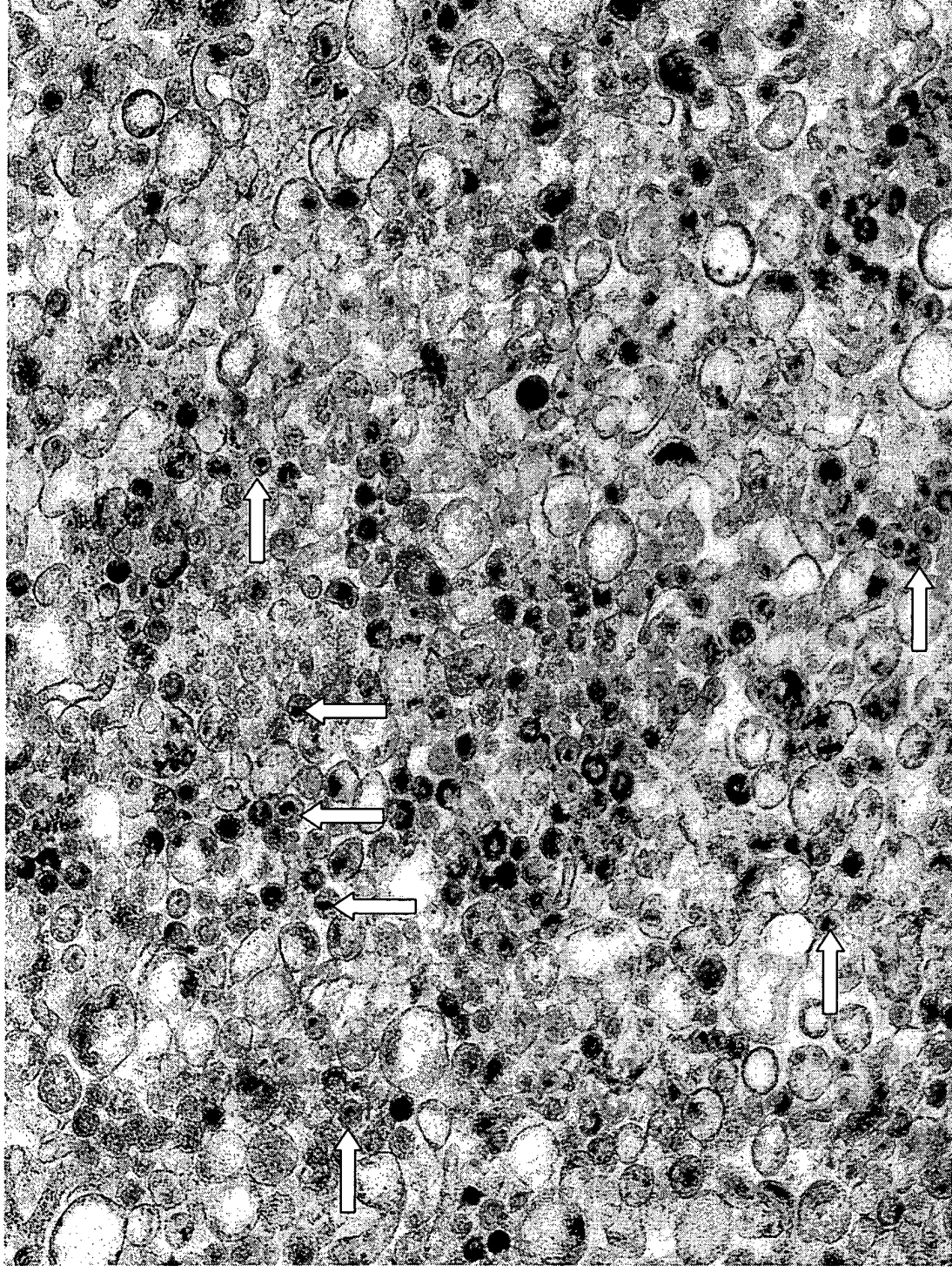


EM Analysis HIV-1RF: 1% Butanol

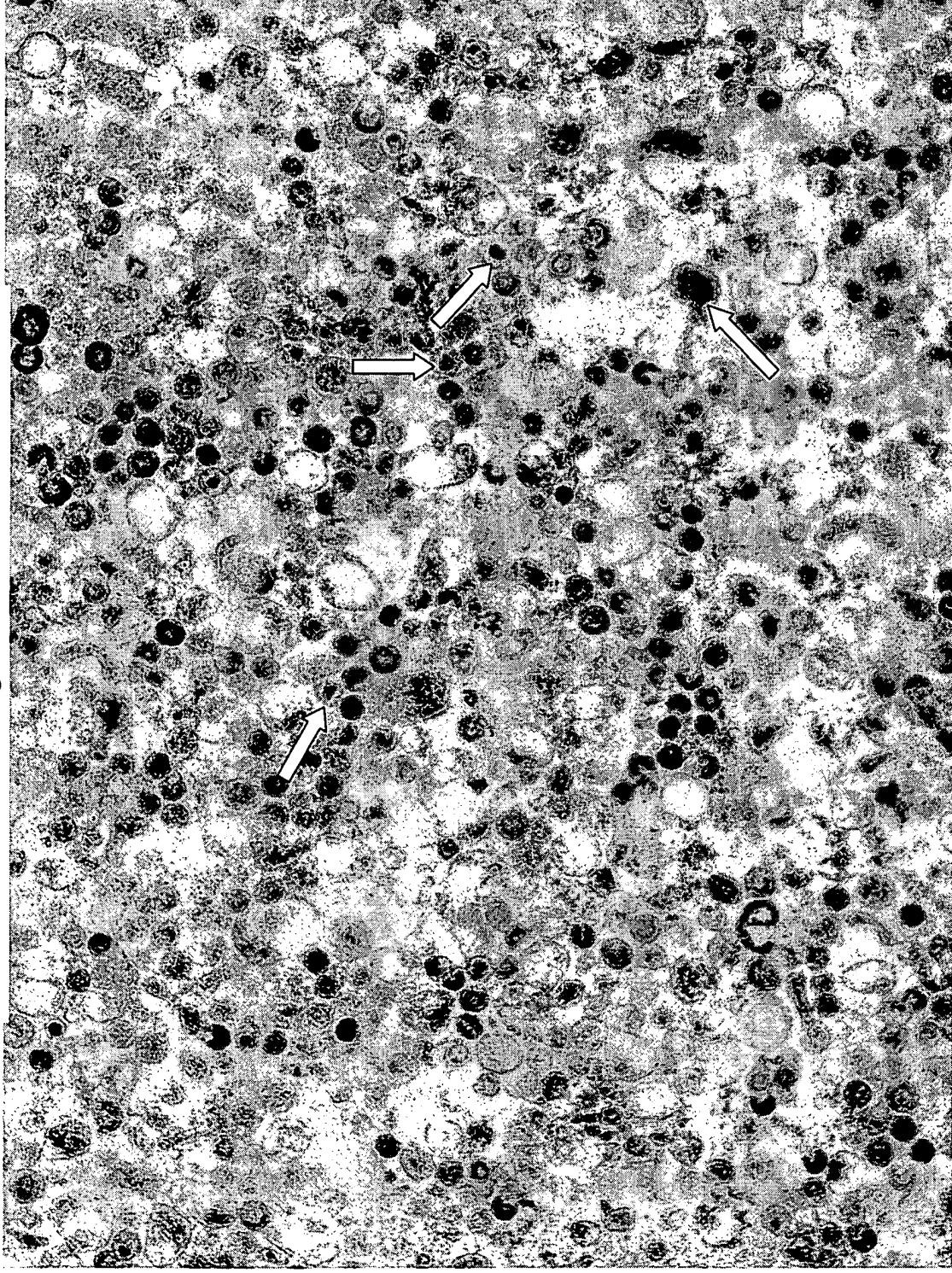
Figure 3



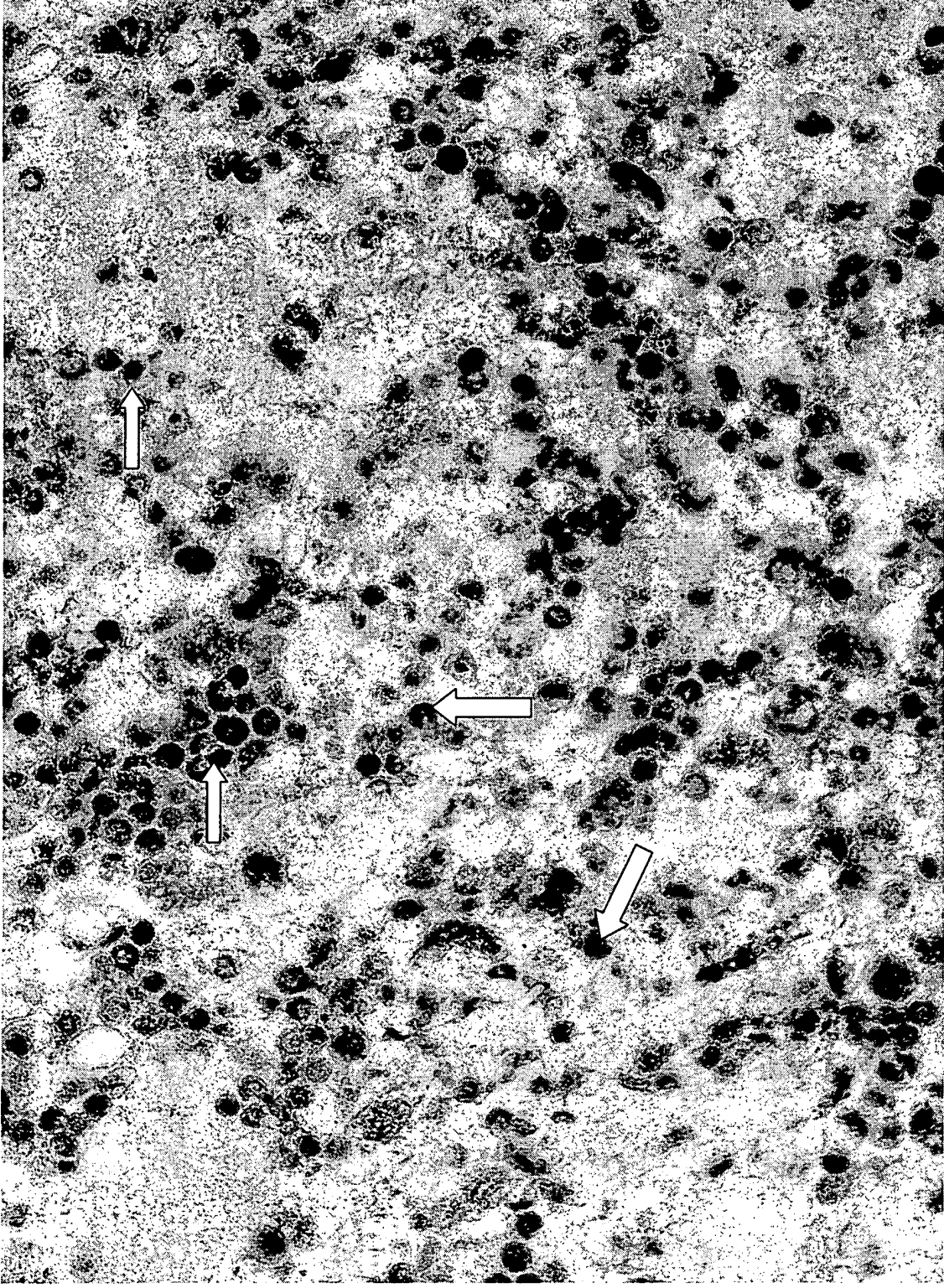
EM Analysis HIV-1RF: 1% Butanol/DIPE
Figure 4



EM Analysis HIV-1RF: 2% Butanol
Figure 5



EM Analysis HIV-1RF: 5% Butanol
Figure 6



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